A Previously Undescribed Nepovirus Isolated from Potato in Peru

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ABSTRACT

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A previously undescribed virus was isolated from a potato plant in central Peru, showing calicolike leaf symptoms. The host range of the virus included a total of 44 species in seven plant families. The virus infected all potato cultivars, and wild potato species inoculated mechanically, but only *Solanum chancayense* and *S. mochisense* were infected systemically. Symptomless systemic infection was established in potato cultivars, however, by graft inoculation. The virus was transmitted readily through seed of *Nicotiana debneyi*, tobacco, *Chenopodium quinoa*, and *C. amaranticolor*. It remained infective in leaf sap at a dilution of 10^{-3} (but not 10^{-4}), when heated at 60 C (but not 65 C), and when stored for 7 (but not 8) days. Sap or purified preparations contained isometric particles ~28 nm in diameter. Purified virus sedimented as three components (top b, middle, and bottom) with sedimentation velocities of 55S, 117S, and 135S, respectively. Occasionally a fourth component (top a) of about 40S was obtained. SDS polyacrylamide electrophoresis yielded a single protein with a molecular weight of 58,000 daltons. The virus was serologically unrelated to 17 nepoviruses, but its properties are typical of this group. We propose that the virus be named potato virus U.

Additional key words: potato virus, serology, nepovirus.

During surveys to identify viruses causing bright yellow leaf markings (calico) in potato fields in the Andean highlands of Peru, a virus isolate (code named UC at the International Potato Center in Lima, Peru [1,2]) was obtained that, when inoculated to *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn., induced symptoms resembling those of the Andean potato calico strain of tobacco ringspot virus (TRSV-Ca) (4,11). Particles viewed in the electron microscope were similar, but no reactions were obtained when UC was tested against antiserum to TRSV-Ca in gel double-diffusion tests (*unpublished*).

The purpose of the detailed investigations reported here was to determine the properties and identity of the virus.

MATERIALS AND METHODS

Isolation and virus cultures. A virus was isolated in 1977 from a potato plant with bright yellow leaf markings (cultivar unknown) growing in the Comas Valley, Department of Junin, central Peru, at an altitude of 3,600 m. It was cultured in plants of *Nicotiana clevelandii* Gray and *N. tabacum* 'Samsun' and 'Xanthi,' and these hosts were used as sources of inocula for the experiments. *N. clevelandii* and *Nicandra physaloides* Gaertn. were used to culture arracacha virus A (AVA) and TRSV-Ca, respectively (4,12).

Plants. Indicator hosts came from seedlings transplanted to pots containing a mixture of sterilized soil, sand, and peat. Wild tuber-bearing *Solanum* spp. were grown from true seed supplied by the USDA Potato Introduction Station, Sturgeon Bay, WI. Some potato cultivars were grown from healthy rooted cuttings supplied by the International Potato Center's seed program and others were grown from healthy tubers supplied by the Department of Agriculture for Scotland, Edinburgh, Scotland.

All experiments were done in greenhouses at 16–20 C. Mechanical inoculations were made by rubbing Carborundum-dusted leaves with cotton swabs dipped in sap inoculum. For studies of properties in infective sap, inoculations were made to groups of three to six plants of *C. amaranticolor* or *C. murale* L.

Electron microscopy. Samples prepared from diluted sap or purified preparations were stained with 2% neutral potassium phosphotungstate and examined in an electron microscope.

Purification, sedimentation behavior, and determination of molecular weight of protein subunits. The virus was purified from infected *N. tabacum* 'Samsun' leaves using the chloroform-butanol procedure used for purification of Andean potato mottle virus (APMV) (5) followed by centrifugation on a 10-40% linear sucrose density gradient (SDG) in a Beckman SW-25.1 rotor. Sedimentation velocities relative to that of tobacco mosaic virus (194S) and the top (53S) and bottom (113S) components of belladonna mottle virus were measured by SDG centrifugation in a Beckman SW-39 rotor. The molecular weight of protein subunits was determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis as described by Koenig et al (14).

Serology. Rabbits were immunized by two intramuscular injections spaced 1 wk apart of a mixture of top, middle, and bottom components of the virus. The virus preparation had a dilution end point of 1:512 in the agar gel double diffusion test. It was emulsified with an equal volume of Freund's complete or incomplete adjuvant for the first or second injection, respectively. Each injection consisted of 2 ml of emulsion. Agar gel double-double diffusion tests were done in plastic petri dishes. Wells were cut 4 mm in diameter at distances of 3 mm. Immuno-electrophoresis was in 1% agarose in 0.025 M phosphate buffer (pH 7.0).

RESULTS

Virus distribution and incidence. All attempts to reisolate the UC virus from potato plants failed. The virus was not detected in 326 potato leaf samples taken at random from different clones of the International Potato Center's germ plasm collection and tested by gel double-diffusion using antiserum prepared to the UC isolate. Neither was it detected in 32 and 28 leaf samples of several cultivars

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with calicolike symptoms collected from different fields in the Comas and Huasahuasi valleys, respectively. These samples were tested by inoculation to *C. quinoa*, *C. amaranticolor*, and *N. clevelandii*, supplemented by serological tests with antisera to the UC isolate and a range of viruses commonly found in Andean potato fields. Other viruses commonly detected in the leaves with calicolike symptoms included APMV, TRSV-Ca, potato mop-top virus, and potato virus X, all of which are known to cause bright yellow leaf symptoms in potato plants growing in the Andean region (4,6,10,11,15,16).

Host range and symptomatology. The UC isolate infected 44 plant species from seven families (Table 1). *Brassica pekinensis* (Lour.) Rupr. (Cruciferae), *Phaseolus vulgaris* L. 'The Prince' (Leguminosae), *Capsicum sinensis* Murr. 'Panca,' and 'Mono rojo' (Solanaceae) developed no symptoms when inoculated with the UC isolate and no virus was detected in them by backtesting to *C. quinoa* and/or *C. murale*.

The most useful diagnostic hosts were *C. quinoa*, which reacted with chlorotic and/or necrotic spots in inoculated leaves, and systemic chlorotic mottle and deformation of young leaves followed by systemic necrosis (Fig. 1A); *C. murale*, necrotic spots in inoculated leaves and systemic necrosis; *C. amaranticolor*, chlorotic and/or necrotic spots in inoculated leaves, and systemic chlorotic mottle and leaf deformation followed by recovery of young leaves produced later (Fig. 1B); *N. tabacum*, systemic chlorotic ringspots and line patterns (Fig. 1C) followed by recovery; and *N. clevelandii*, a faint mosaic followed by recovery. Characteristic symptoms also developed in *Beta vulgaris* L. 'Red Beet' consisting of red rings in inoculated leaves and systemic red vein banding with some systemic red rings, spots, and blotches. *Cucumis sativus* developed local chlorotic blotches in inoculated leaves and systemic vein clearing, chlorotic blotching, and mosaic followed by recovery.

Of the eight wild tuber-bearing *Solanum* spp. tested, only *S. chancayense* Ochoa and *S. mochicense* Ochoa became infected systemically, producing chlorotic blotching and mosaic. In contrast, all eight became infected in inoculated leaves, developing diffuse necrotic rings and dots, chlorotic blotches, or symptomless infection (Table 1). None of 12 cultivars and two clones of cultivated potato became infected systemically when inoculated mechanically with the UC isolate, but all were infected in inoculated leaves. Their inoculated leaves were infected symptomlessly, or developed necrotic spots or rings (Fig. 1D), or dark-green blotches or rings appeared when they became senescent. In attempts to establish systemic infection with the UC isolate, plants of *S. tuberosum* 'Desirée,' 'Maris Piper,' 'Home Guard,'

TABLE 1. Symptomatology of UC virus in indicator hosts, wild potato species, and potato cultivars

Species	Symptoms[a]	Species	Symptoms[a]
Aizoaceae		*Petunia hybrida* Vilm.	SVC, R
Tetragonia expansa Murr.	SM, LD	*Physalis floridana* Rydb.	MM
Amaranthaceae		*P. peruviana* L.	LCR, SVC, SCR, R
Gomphrena globosa L.	LRS, SVC, R	*Solanum berthaultii* Hawkes (P.I. 265857)[b]	SI, NS
Chenopodiaceae		*S. chacoense* Bitt. (P.I. 275136)	SI, NS
Beta vulgaris L. 'red beet'	LRR, RVB, SRS, SRR	*S. chancayense* Ochoa (P.I. 338615)	LNS, LNR, SM, SCS, LD
B. vulgaris L. 'sugar beet'	LNR, LCR, CVB, R	*S. demissum* Lindl. 'A'	SI, NS
Chenopodium album L.	LCS, LCR, SM, LD, R	*S. demissum* × *S. tuberosum* L. 'A6'	LCS, LCR, NS
C. amaranticolor Coste & Reyn.	LNS, LCS, SM, LD, R	*S. goniocalyx* Juz. & Buk. 'Huayro'	SI, NS
C. capitatum (L.) Asch.	SS	*S. megistacrolobum* Bitt. (P.I. 263873)	SI, NS
C. ficifolium Sm.	LCS, LCR, SM, SVC, LD, R	*S. microdontum* Bitt. (P.I. 218223)	LNS, LNR, LCS, NS
C. foliosum (Moench) Asch.	MM, SCS, R	*S. mochisense* Ochoa (P.I. 283114)	LCS, LCR, MM, SCS, SCR, SVC, R
C. glaucum L.	LCS, LCR, SS	*S. stoloniferum* Schlecht. (P.I. 230557)	SI, NS
C. murale L.	LNS, SN	*S. tuberosum* subsp. *andigena* Juz. & Buk.	
C. quinoa Willd.	LNS, LCS, SM, LD, SN	'Ccompis'	LNR, NS
Spinacea oleracea L.	SS	'Renacimiento'	LGS, LGR, NS
Cucurbitaceae		'Sipeña'	LNS, LNR, NS
Cucumis sativus L.	LCS, SM, SVC, SCS, R	*S. tuberosum* L. × *andigena*	
Leguminoseae		'Mariva'	SI, NS
Vigna cylindrica Skeels	SS	'Ranrahirca'	LNS, NS
V. sinensis (Torn.) Savi	SS	*S. tuberosum*	
Portulacaceae		'Desirée'	SI, NS
Montia perfoliata (Willd.) Howell	SS	'Home Guard'	SI, NS
Solanaceae		'Maris Piper'	LNR, LGR, NS
Datura metel L.	SCR, SCL, R	'Pentland Crown'	SI, NS
D. stramonium L.	LCR, SCR, SCL, R	'Pentland Dell'	LNR, NS
Hyoscyamus niger L.	SI, NS	'Record'	SI, NS
Lycopersicon chilense Dun.	SI, NS	'USDA 41956'	SI, NS
L. pimpinellifolium (Jusl.) Mill	SCR, SCL, R	*S. villosum* Lam.	LCS, LCR, SCS, SCR, SCL, R
L. esculentum Mill. 'Rutgers'	SS		
Nicandra physaloides Gaertn.	LNS, NS		
Nicotiana bigelovii Wats	MM, R		
N. clevelandii Gray	MM, R		
N. debneyi Domin	MM, R		
N. glutinosa L.	SS		
N. occidentalis Wheeler	LNR, SNL, SCL, R		
N. rustica L.	SS		
N. tabacum L. 'Samsun,' 'White Burley,' and 'Xanthi'	SCR, SCL, R		

[a] Coded symptom descriptions: LCS = local chlorotic spots or blotches; LCR = local chlorotic rings or ringspots; LGS = local green spots in yellowed senescent leaves; LGR = local green rings in yellowed senescent leaves; LRS = local red spots; LRR = local red rings; LNS = local necrotic spots; LNR = local necrotic rings or ringspots; SI = symptomless infection in inoculated leaves; NS = no systemic infection; MM = mild or faint mosaic; SM = strong mosaic or chlorotic mottle; SVC = systemic vein clearing; SCS = systemic chlorotic spots or blotches; SCR = systemic chlorotic rings or ringspots; SCL = systemic chlorotic line patterns; SNL = systemic necrotic line patterns; SRS = systemic red spots or blotches; SRR = systemic red rings; SN = systemic necrosis; LD = systemic deformation of young leaves; CVB = systemic chlorotic vein banding; RVB = systemic red vein banding; R = recovery in young leaves produced later; and SS = symptomless systemic infection.

[b] Numbers in parentheses are plant introduction numbers, USDA Potato Introduction Station, Sturgeon Bay, WI, USA.

'Pentland Crown,' and 'Pentland Dell' were either mechanically inoculated on the stem or top-grafted with scions of infected tobacco and/or *N. debneyi* Domin., but virus was not detected by backtesting to *C. murale*. Similarly, no infection could be detected in scions of these cultivars top-grafted onto UC virus-infected tomato plants. The virus was recovered, however, from scions of 'Desirée,' 'Maris Piper,' 'Pentland Dell,' and 'Pentland Crown' top-grafted onto infected *N. tabacum* 'Xanthi' plants, but only after repeated backtesting, indicating a low virus concentration. No symptoms were associated with this infection. By contrast, calico-

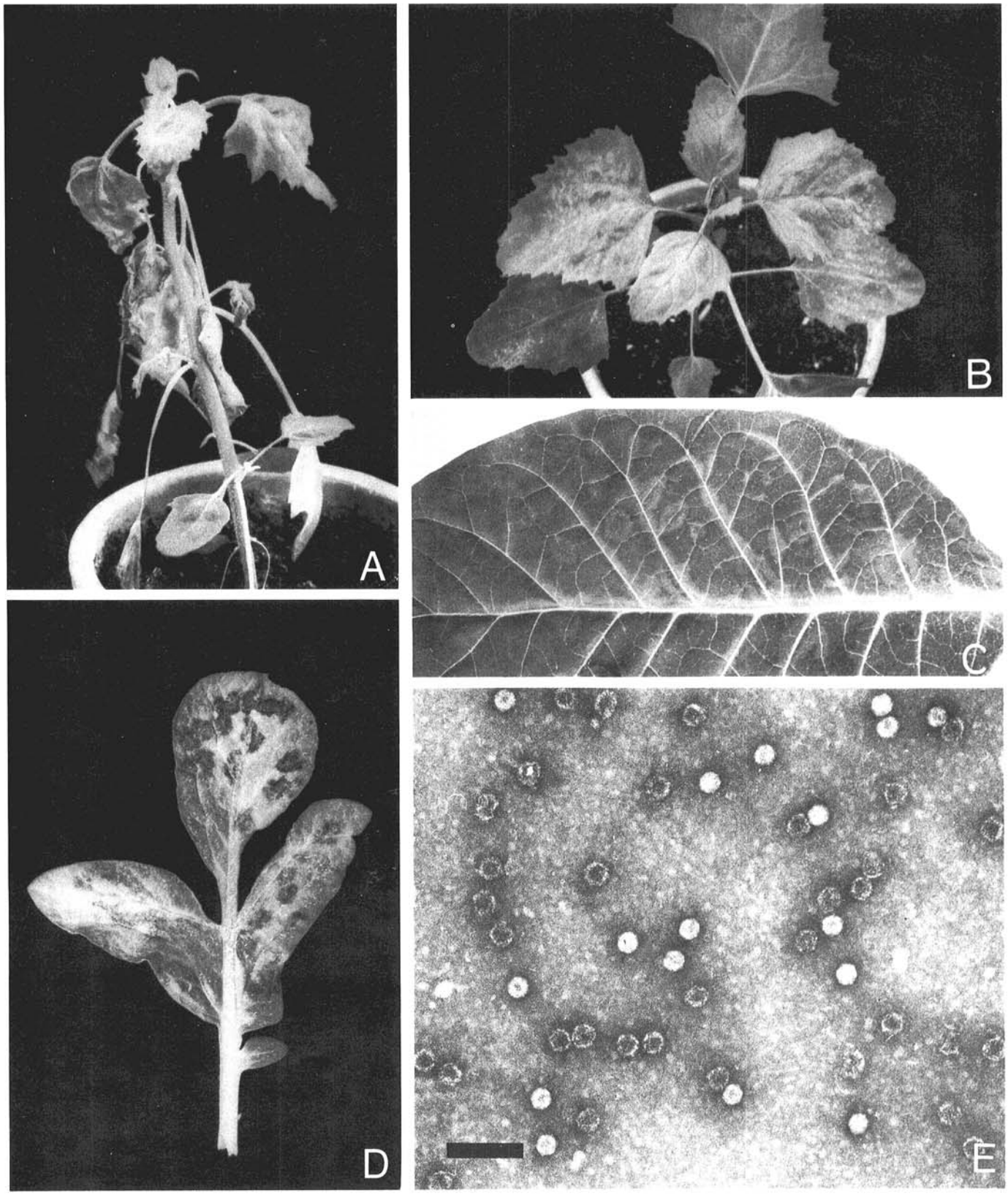

Fig. 1. Symptomatology and electron microscopy of UC virus. **A,** Systemic chlorotic mottle, leaf deformation and necrosis in *Chenopodium quinoa*. **B,** Local lesions and systemic chlorotic mottle in *C. amaranticolor*. **C,** Systemic chlorotic rings and line patterns in *Nicotiana tabacum* 'Samsun.' **D,** Necrotic local lesions in *Solanum tuberosum* 'Sipeña.' **E,** Electron micrograph of virus particles from a partially purified preparation. Bar = 100 nm.

like symptoms resembling those in the potato plant from which the UC virus was originally isolated were readily reproduced when tubers harvested from TRSV-Ca inoculated plants of cultivar Ticahuasi were grown under greenhouse conditions; this other nepovirus is commonly associated with such symptoms in the field in the Andes (4).

Seed and aphid transmission. Apterous *Myzus persicae* Sulz. were allowed to feed for either 1 min (three experiments) or 3 days (four experiments) on UC virus-infected *C. quinoa* or *N. clevelandii* and then for either 1 hr or 3 days, respectively, on healthy plants of *C. murale* (five aphids per plant). In each of the seven experiments, none of the 10 test plants became infected. By contrast, the virus was readily transmitted through seed of indicator hosts. Thus, when seedlings grown from seed collected from UC virus-infected plants of *C. quinoa* and *C. amaranticolor* were tested individually by inoculations to *C. murale* or *C. quinoa*, infection was detected in 66/100 and 67/90 seedlings, respectively. Similarly, when seedlings grown from UC virus-infected plants of *N. debneyi* and *N. tabacum* 'Xanthi' were tested, the virus was detected in 8/20 and 14/20 batches of five seedlings each, respectively. By using the formula of Gibbs and Gower (7) the percentage of seed infected was estimated to be 10% for *N. debneyi* and 21% for *N. tabacum* 'Xanthi.'

Properties in sap. Infectivity in sap of *N. clevelandii* was lost by diluting 10^{-4}, but not 10^{-3}, in distilled water; by heating for 10 min at 65 C, but not at 60 C; and by storage at about 20 C for 8 days, but not for 7 days. In *C. quinoa* leaves desiccated and held over silica gel, infectivity was still maintained after at least 1.5 yr.

Electron microscopy. Expressed sap from infected plants and purified preparations contained isometric particles about 28 nm in diameter with a hexagonal profile, some of which were either fully or partially penetrated by the negative stain (Fig. 1E). Most particles appeared to be slightly damaged.

Sedimentation behavior. In SDG, UC virus usually sedimented as three components with sedimentation velocities of 135S (bottom), 117S (middle), 55S (top b); occasionally a fourth component (top a) with a sedimentation velocity of about 40S also was obtained.

Molecular weight of protein subunits. SDS polyacrylamide electrophoresis yielded a molecular weight of 58,000 daltons (average of four determinations) when bovine serum albumin (67,000), heavy (50,000) and light (23,500) chains of γ-globulin, fumarase (49,000), egg albumin (43,000), alcohol dehydrogenase (37,000), tobacco mosaic virus coat protein (17,800), and lysozyme (14,300) were used as marker proteins.

Serology. Antiserum to UC virus had a homologous titer of 1:256 and did not react with healthy *N. tabacum* 'Samsun' sap nor with TRSV-Ca or AVA. UC virus-infected *N. tabacum* 'Samsun' sap did not react with antisera to TRSV-Ca or AVA, or to arabis mosaic, artichoke Italian latent, artichoke vein banding, cherry leaf roll, cocoa necrosis, grapevine Bulgarian latent, grapevine chrome mosaic, grapevine fanleaf, hibiscus latent ringspot, myrobalan latent ringspot, olive ringspot, raspberry ringspot, strawberry latent ringspot, tobacco ringspot (NC-38 and eucharis mottle strains), tomato black ring (beet, bouquet, and potato pseudoaucuba strains), and tomato ringspot.

In immunoelectrophoresis at pH 7.0, UC virus migrated towards the anode.

DISCUSSION

UC virus resembles the nepoviruses (3,9) in its particle morphology, sedimentation behavior, protein subunit molecular weight, and properties in sap. Moreover, like other nepoviruses, it has a wide host range, can cause systemic ringspot symptoms in some hosts and is readily seed-transmitted. In preliminary experiments, it was transmitted by an unknown species of the nematode *Longidorus* (P. Jatala and C. E. Fribourg, *unpublished*). The only nepoviruses previously reported from Peru are the eucharis mottle strain of TRSV (13), TRSV-Ca (4), and AVA (12). No serological reaction was detected when UC virus was tested with antisera to these viruses or to 15 other different nepoviruses.

The UC virus was only once found naturally infecting potato and systemic infection of potato cultivars could only be established with difficulty, by top grafting potato scions onto UC virus-infected tobacco plants. The virus, therefore, may be poorly adapted to this host, only occasionally spreading to it from other as yet unknown natural host species. Alternatively, like tobacco rattle virus (8,11), it may predominantly infect potato roots, stolons, and tubers, only occasionally moving systemically to the foliage.

No attempt was made to look for UC virus naturally infecting other hosts in the field. However, under greenhouse conditions the virus causes a severe disease of *C. quinoa*, which is a common grain crop at high altitude in the Andes, and also infects beet, spinach, cucumber, tomato, tobacco, and cowpea, which are grown at low altitude. Weed hosts of UC virus in the Andean region, which were infected experimentally, include: *Physalis peruviana* L., *Lycopersicon pimpinellifolium* (Jusl.) Mill., *Datura stramonium* L., *Datura metel* L., and several *Nicotiana* spp. The virus may, therefore, be widespread in weeds and certain crop plants. Seed transmission could be an important means of spread in them; UC virus was efficiently seed-transmitted in the *Chenopodium* and *Nicotiana* spp. tested. Further work is needed to provide information on its distribution in hosts other than potato and on spread in the soil by its nematode vector. The name potato virus U is proposed.

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